Cover Page

Proposal to:
California Department of Food and Agriculture
Pest Exclusion Branch/Nursery, Seed and Cotton program
Attn: Katherine Filippini
1220 N Street
Sacramento, CA 95814

Submitting Organization: University of California Riverside

Project Title: Development and validation of virulence markers for vineyard phylloxera

Project Period: Year: One Year 07/01/2021-06/30/2022

Amount Requested: $ 33,014

Principal Investigators:

Dr. Paul Nabity
Assistant Professor of Plant-Insect Ecology
Dept. of Botany and Plant Sciences
UC Riverside
900 University Ave
Riverside, CA 92521
Paul.nabity@ucr.edu – (951) 827-3927

Cooperating Personnel: None

Checks Made Payable to: The Regents of the university of California

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Main Cashier's Office
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Student Services, Bldg., Room 1111
Riverside, CA 92521
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Project Summary and Scope of Work

Project Title: Development and validation of virulence markers for vineyard phylloxera

Executive Summary

The grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), remains a challenge to viticulture because of the possibility for new genotypes to evolve or invade. Indeed, sexual forms have appeared in Northern California with evidence of genetic recombination resulting in new insect genotypes [1] and new invasions have recently occurred and become established in Washington State. When these insects adapt to rootstocks, viticulturists face significant management costs to reduce pest populations, ultimately replanting self-rooted or susceptible rootstocks with new rootstocks. **How grapes tolerate or resist phylloxera remains unknown** and this limits the breadth of rootstocks available because a first step in any rootstock deployment is to select from phylloxera resistant genotypes before considering other factors (e.g., disease resistance or tolerance to drought or salinity). To advance our understanding of what enables phylloxera to feed and adapt to rootstocks this project will leverage a recent international genome sequencing effort and preliminary data on phylloxera responses across wild germplasm used in rootstock breeding to apply this information to current rootstocks. Specifically we will 1) Determine the performance-based gene expression of phylloxera clones across select rootstocks, 2) Identify genotyping targets of functional importance to improve field assessments of phylloxera virulence, 3) Validate this predictive framework on historic samples, and 4) Disseminate research results through open access venues. Objectives 1 and 2 will be completed by normalizing nucleic acid expression and change to a performance assay following standardized techniques. The predictive framework resulting from these experiments will be validated by fully genotyping historical samples and disseminated via open access publications. This information will establish a set of genetic markers linked to phylloxera virulence to 1) enable prediction of phylloxera adaptability given rootstock plantings and vineyard phylloxera populations, and 2) aid in rootstock screening trials to increase the number of rootstock available.

Project's Benefits to the Nursery Industry

How phylloxera overcomes rootstock resistance remains unknown. Because no rootstocks can be grown without first demonstrating some resistance to phylloxera (to avoid risk), understanding what enables phylloxera to feed and how phylloxera adapts to rootstocks are crucial to developing long-term and vineyard-specific management strategies. The international collaboration that sequenced the grape phylloxera genome found over 2300 genes that are active during feeding on grape and share similar structure to genes known as effectors that allow the insect to overcome grape immune/defense pathways [2]. These effector genes were largely annotated by my lab and additional characterization confirms they engage with grape immune and defense pathways through processes shared among plants [3]. Unpublished data highlight select insect genes that are more highly or lowly expressed during incompatible interactions, thereby providing candidates for functional proteomics assays now underway. To further this research and apply it to rootstock breeding, we need to know if these genes show variation in expression or are targets of epigenetic change. In other words, are these insect genes actively changing when the insect experiences a new rootstock with wild grape resistance in its genetic background? This would tell us if and how fast phylloxera are evolving in vineyards. As a trial of this approach, we aim to use commonly planted rootstocks in a performance-based gene expression and epigenetic assessment. Although this project is primarily research, we will identify which genes in phylloxera are under pressure to change when encountering new hosts/rootstocks (Obj 1). This will allow us to develop new genotyping strategies to assess the adaptability of populations existing within vineyards (Obj 2 and Obj 3). We will also identify how phylloxera adapts to contemporary rootstocks widely used in the US. This research aligns with the goals of IAB by providing fundamental knowledge toward a long-

term solution of grape phylloxera management. Because no information exists on the insect genes that enable phylloxera to adapt to new genotypes, this project also provides a tangible output for growers. Predicting outbreaks or determining if existing phylloxera populations will increase in virulence with new plantings requires time intensive screens with local genotypes. By identifying which genes are most active and under greatest pressure to evolve, this project will link performance (and ultimately virulence) to insect genes to begin building a virulence map on cultivated and wild hosts. This will enable rapid genotyping of existing phylloxera populations to predict what rootstocks will grow better in the individual vineyard and the identification of hot spots where quarantines may be necessary. Currently, phylloxera genotyping assays enable us to identify where individuals invade from but we do not know what enables invasion or how genotypes adapt in the vineyards. Because rootstock parentage is the main factor used when planting or replanting in phylloxera-prone areas, this project will also aid rootstock selection by linking plant heritage to phylloxera genes of known function and directly related to performance. **Such matching will enable more choices in rootstock deployment when other traits are more desirable (e.g., drought, salt, or nematode tolerance).** It may seem counterintuitive, but a better understanding of the insect will allow us to develop and implement more rootstocks across variable environmental settings.

Objectives

1. Determine the performance based gene expression of phylloxera clones across rootstocks.
2. Identify genotyping targets of functional importance to improve field assessments of phylloxera diversity.
3. Validate this predictive framework on historic samples.
4. Disseminate research results through open access venues.

Workplans and Methods

1. Determine the performance based gene expression of phylloxera clones across rootstocks.

Whole plant grapevines of rootstocks (110R, 1103P, 101-14, 3309, and 1616C) and wild species (*V. vinifera* pinot meunier and *V. riparia*) will be grown in an environmental growth chamber (25:22 ˚C day:night with 16h daylength). Phylloxera will be field collected from NCGR, Davis, CA, and a clonal colony established on susceptible *V. vinifera* pinot meunier. Root enclosion vessels (modified petri dishes) will be infested with 50 eggs of clonal phylloxera following typical bioassay protocols for phylloxera [4]. Colonization and survival will be monitored every two days until insects reach adult and begin laying eggs. Eggs will be counted and removed daily for the following 10d. This performance and fecundity trial will be analyzed using the ‘survival’ and ‘survminer’ packages in R with Bonferroni-Hochberg adjusted p-values. This trial will be repeated to collect insects for genetic profiling just before performance declines or at 20d post infestation, whichever comes first.

Once the proper collection time is identified, pools of insects per plant genotype will be collected in replicate and profiled following [2]. Independent plants will be combined for each pool with four biological replicates/pools created for each genotype. RNA will be extracted following kit protocols and libraries will be prepared at the UCR Genomics Core prior to sequencing. Read output will be cleaned (Bbduk;[5]) and aligned to the genome (using STAR; [6]) to generate gene counts. Differential expression between genotypes will be assessed using EdgeR [7,8].

DNA will be collected from egg laying adults to maximize the potential for methylation within a single generation. DNA will be bisulfite treated and prepped for sequencing following kit protocols. Reads will be cleaned and mapped as above. Methylation differences among genotypes will be assessed using principal component analysis and logistic regression with multiple testing correction [9].

2. Identify genotyping targets of functional importance to improve field assessments of phylloxera diversity.

Preliminary investigation of phylloxera gene expression profiles showed several important functional groups lacked expression of genes in populations that are incompatible (100% mortality) with wild species used in rootstock breeding. For example, 1) 260 effector genes were not expressed in phylloxera failing to colonize *V. riparia*, including 7 genes recently identified as important to feeding on leaves of *V. riparia* and both leaves and roots of *V. vinifera* Cabernet Sauvignon [3]; and 2) 12 genes related to detoxifying plant defenses were not expressed. These data support **the hypothesis that lack of or low expression of genes impairs phylloxera in overcoming plant immune responses**. Evidence exists in aphids that both presence and increased expression of effectors contribute to host adaptation [10], and overexpression of a single detoxification gene confers xenobiotic resistance in other insects [11], setting precedence for single genes to control resistance through expression. Ultimately, this can lead to adaptation in field populations. Phylloxera retain all the genes necessary for epigenetic adaptation, or heritable genetic change within a single generation [2]. Therefore, gene expression and epigenetic profiling will be correlated with genes previously identified as active during feeding or classified as effectors contribute to phylloxera virulence. To link genes of interest in Obj1 to their function, we will use an updated, soon-to-be published chromosome-level genome assembly of phylloxera. Methylated and differentially expressed genes will be inspected for structure and function (e.g., candidate effector, detoxification, sensory) based on new genome annotations.

3. Validate this predictive framework on historic samples.

Genes identified as important to feeding on rootstocks by Obj 1 and 2 will be screened for variation in field samples previously collected by Dr. Andy Walker [12]. A subset (30) of the 500 samples from A. Walker will be genotyped for single nucleotide polymorphisms (SNPs) following standard DNA library preparation and sequencing at the UCR genomics core. Samples will include rootstocks from CA vineyards and wild species. Reads will be generated at a genome coverage ~30x and will be cleaned and aligned using software described above with SNPs called following standard protocols [13]. These data will show if genes identified in Obj 1 and 2 are variable in the field to link expression and epigenetic change with adaptation to rootstocks. A preliminary map of virulence will be constructed. If these data support the predictive framework, more samples can be evaluated in future trials.

4. Disseminate research results through open access venues.

Research will be presented at appropriate extension and growers conferences. Data will be published with open access and provided freely on lab webpages.

Project Management and Evaluation

The PI will plan and oversee setup, data collection, and analysis. The PI will also prepare reports for IAB and manuscripts for publication. Dr. Walker has agreed to provide archived specimens. Two postdoctoral scientists in the lab will also contribute to setup, data collection and analysis, and manuscript writeup. The graduate student involved will also contribute to data collection, analysis, and writeup. Success will be determined by: **Colonization/performance**. Length of survival on rootstocks and time of reproduction will serve as a baseline for sample collection for RNA. **Insect longevity**. Insects reproduction will indicate mature adults and eggs can be collected for DNA. Sample preparaition and sequencing will take 1-2 months. Data analysis is streamlined and will commence rapidly once data are generated. If per sample DNA from archived samples is low, samples will be pooled per location.

Timeline	Deliverable
Summer 2021	Colony established, Performance trial, DNAseq of historic samples and for epigenetic change after performance trial
Fall 2021	RNAseq of repeated performance trials. Progress report submitted
Winter 2021	Data analysis, Preliminary map
Spring 2021	Data analysis, writeup, dissemination. Final report submitted

Literature Review

How do insect pests adapt to crops? This question remains outstanding for most plant-insect combinations. Perennial, clonally propagated crops, such as grapes, have long generation times and reduced genetic diversity that enable pests to rapidly adapt to plant defenses. Because insects, especially aphid-like insects such as phylloxera use salivary secretions to help evade or misdirect the plant immune system, creating long-term solutions to pests must rely on knowledge of both the plant and insect. The genome of grape phylloxera identified over 2300 genes that are structurally similar to effector genes and enable feeding across grape species with various resistance traits [3]. Related insects such as aphids are known to show differential gene expression of effector-like genes [10] when colonizing new hosts, and use epigenetic machinery to enable genetic change in clonal lineages in response to their environment [9]. Therefore it is likely grape phylloxera can adapt similarly over time and the means by which adaptation occurs can be identified and used to predict virulent host interactions. When local vineyard adaptation is combined with the potential for invasion of or genetic recombination within vineyards, rootstock longevity becomes diminished.

Literature Cited

1. Riaz, S., Lund, K. T., Granett, J. & Walker, M. A. Population Diversity of Grape Phylloxera in California and Evidence for Sexual Reproduction. *Am J Enol Viticult* **68**, 218–227 (2017).

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3. Zhao, C., Rispe, C. & Nabity, P. D. Secretory RING finger proteins function as effectors in a grapevine galling insect. *Bmc Genomics* **20**, 923 (2019).

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11. Daborn, P. J. *et al.* A Single P450 Allele Associated with Insecticide Resistance in Drosophila. *Science* **297**, 2253–2256 (2002).

12. Lund, K. T., Riaz, S. & Walker, M. A. Population Structure, Diversity and Reproductive Mode of the Grape Phylloxera (Daktulosphaira vitifoliae) across Its Native Range. *Plos One* **12**, e0170678 (2017).

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IAB – Budget Proposal

Project Title/Description: Development and validation of virulence markers for vineyard phylloxera

Project Leader: Paul Nabity

Proposed Fiscal Year: 2021

A. PERSONNEL SERVICES:	
Classification	
GSR IV @ 4596.92 monthly@ 49%, FTE, 1 quarter	$6,960
Staff Benefits @ 1.8%	$125
Graduate Student Health Insurance and Partial Fee Remission	$5,909
TOTAL PERSONNEL SERVICES:	$12,994
B. OPERATING EXPENSES:	
Laboratory Supplies	
-RNA extraction kit	$ 250
-DNA methylation kit	$2,800
Services	
RNA sequencing	
(library prep and sequencing = $210/sample)	
(4 samples/genotype * 7 genotypes)	$5,880
Methylation DNA sequencing	
($140/sample 3 samples/genotype * 7 genotypes)	$2,940
SNP DNA sequencing	
(library prep and sequencing = $195/sample, 30 samples)	$5,850
Computational support	
Data storage	$1,000
TOTAL OPERATING EXPENSES:	$18,720
C. INDIRECT COST:	$1,299
D. TOTAL BUDGET REQUESTED:	$33,014

*Round dollar amount to the nearest dollar
*Type out acronym “FTE”
*Make sure % and dollar amount add up

Budget Justification

Key/Senior Personnel
Paul Nabity, Principal Investigator:
No funds requested.

Other Personnel
Staff Classification:
Graduate Student Researcher (GSR) IV - Salary support is requested for one graduate student at 49% FTE for one quarter, based on a monthly rate of $4,596.92 per month. The GSR will will work on effector validation and virulence genetics analyses.

Fringe Benefits
Fringe benefits rates are calculated as a percentage of the gross salary using University approved composite rates and are as follows: The GSR percentage is 1.8%, totaling $125.

Additional benefits for GSR also include Graduate Student Health Insurance (GSHIP), Partial Fee Remission (PFR) for one quarter. Based on University policy, tuition and fees are mandatory when the student is a GSR for more than 25% of the academic quarter. The benefit rates used are in accordance with the rates reported to our audit agency, DHHS. The GSHIP/PFR requested is $5,909.

Travel
None requested

Materials and Supplies
Reagents and supplies for extracting nucleic acids are requested at $3,050.

Other Costs
Sequencing at the UCR genomics core will require library preparation of samples and nucleic acid sequencing on Novaseq at negotiated rates totaling $14,670.
To provide access to and storage on the UCR computing core, a $1000 extra data storage fee is requested for the duration of the project.

Indirect Costs
10% of personnel costs ($1,299) are requested as the maximal allowable IDC.